Prevalence and antimicrobial resistance profile of *Campylobacter* isolated from conventional and antibiotic free swine farms in three geographic locations

Daniel A. Tadesse[1], Julie A. Funk[2], Peter B. Bahnson[3], W. E. Morgan Morrow[4], Siddhartha Thakur[5] and Wondwossen A. Gebreyes[1*]

[1]Dept. of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, 1920 Coffey Road, Columbus OH, USA
[2]National Food Safety and Toxicology Centre, College of Veterinary Medicine, Michigan State University
[3]Dept. of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison
[4]North Carolina State University - Department of Animal Science, [5]Food and Drug Administration, Center for Veterinary Medicine

* *Corresponding author:gebreyes.1@osu.edu*

Abstract

Background: The prevalence and antimicrobial resistance of *Campylobacter* were examined from swine reared in conventional and antimicrobial-free (ABF) production systems in three geographical locations: North Carolina (NC), Ohio (OH) and Wisconsin (WI).
Methods: A total of 1500 pigs and 1930 carcass swab samples were evaluated for the prevalence of *Campylobacter*. Fecal samples from 662 pigs from NC (370 conventional farms and 292 ABF farms), 379 from OH (268 conventional and 111 ABF) and 459 from WI (160 conventional and 299 ABF) were included. Antimicrobial susceptibility testing was performed using agar dilution method against a panel of six antimicrobials.
Results: *Campylobacter* was commonly found in swine herds in all the three states with a prevalence of 54.2% (NC), 54.1% (OH) and 58.2% (WI). The prevalence of *Campylobacter* in conventional farms (56.1%) was not significantly different from ABF farms (54.6%) ($p > 0.05$). However, the prevalence of *Campylobacter* in WI farms was higher in conventional farms (70.6%) than ABF (51.5%) ($p<0.05$). *Campylobacter* isolates showed resistance to all the six antimicrobials with different frequency. Tetracycline resistance was the most common followed by erythromycin in all the three states. Frequency of resistance to ciprofloxacin (MIC of >4 mg/L) was higher among OH and WI isolates than NC. Erythromycin and tetracycline resistance was significantly higher in conventional farms than ABF Farms ($p<0.05$). Ciprofloxacin and Nalidixic acid resistance was more common in ABF farms ($p<0.05$). The predominant resistance patterns were erythromycin-tetracycline (Ery-Tet) and tetracycline only (Tet).
Discussion: This study showed high prevalence of *Campylobacter* among swine herds in the three states with no significant difference by geographical locations and production systems (regardless of the antimicrobial use status). The high proportion of ciprofloxacin resistant isolates from ABF herds may have important implications on the potential role of risk factors other than mere antimicrobial use for production purposes and probably producer compliance.

Introduction

Campylobacter is one of the leading causes of foodborne bacterial infection worldwide and an estimated more than 2 million cases of *Campylobacter*iosis occur each year in the United States (Mead et al., 1999). The overall incidence rate of laboratory-confirmed *Campylobacter* infection in 2005 in the United States was 12.72 cases per 100,000 populations (CDC, 2006). Most *Campylobacter* infections in human are self-limited and do not require antimicrobial therapy unless there is some complication. The drugs of choice in such conditions are erythromycin and fluoroquinolones. There are reports of an increase resistance to tetracycline, erythromycin and fluoroquinolones (Prats et al., 2000; Engberg et al., 2001; Gupta et al., 2004; Thakur and Gebreyes, 2005). There are concerns over the transfer of antimicrobial resistance from food animals to human population as animal products can serve as a source of foodborne infection and important link between animals and humans. The use of antimicrobials for growth promotion and prophylaxis in animal industry exacerbate the conditions. The present study investigated the

prevalence and antimicrobial resistance of *Campylobacter* recovered from swine reared under conventional and antibiotic free (ABF) production systems at farm and slaughter from three different geographic locations.

Materials and Methods

Samples were collected from 55 farm-slaughter pairs from conventional and antibiotic free (ABF) swine farms and slaughter plants from three geographic locations: 20 from North Carolina (10 conventional and 10 antimicrobial free farms (ABF)), 16 sites from Ohio (9 conventional and 7 ABF) and 19 sites from Wisconsin (6 conventional and 13 ABF). A total of 1500 fecal and 1930 carcass samples were examined for the presence of *Campylobacter* from North Carolina (662 fecal and 757 carcass swabs), Wisconsin (459 fecal and 680 carcass swabs) and Ohio (379 fecal and 493 carcass swabs). In conventional production system, antimicrobials were used for treatment and growth promotion purposes. In the antibiotic free swine rearing system, antimicrobials were not used after weaning age either for treatment or growth promotion purposes.

Fecal samples were collected from the rectum of live swine and directly plated on campy-cefex selective plates at 42°C for 48 hrs under microaerophilic conditions (10% C_2O, 5% O_2 and 85% N_2). Putative colonies were transferred to Muller Hinton agar (Remel, USA) and tested by catalase (Becton Dickinson, USA) and oxidase tests (Becton Dickinson, USA). Carcass swabs were collected from slaughter plants at different stage of processing pre- and post-evisceration and chill stages using multiple swipe (USDA) and single swipe carcass swabbing methods. Then carcass swabs were enriched in 30 ml Bolton broth (Oxoid, Hampshire, UK) and incubated at 42°C for 48 hrs in microaerophilic conditions. A loopful of the enriched sample were streaked onto campy-cefex and processed similar to fecal samples. Catalase and oxidase positive isolates were tested for antimicrobial susceptibility using agar dilution method against six antimicrobials following the recommendation of the Clinical and Laboratory Standard Institute (CLSI). The antimicrobials and break points used were chloramphenicol (Ch: 0.25 to 128 mg/liter, 32 mg/liter), erythromycin (Ery: 0.06 to 32 mg/liter, 8 mg/liter), gentamicin (Gen: 0.06 to 32 mg/liter, 16 mg/liter), ciprofloxacin (Cip: 0.008 to 4 mg/liter, 4 mg/liter), nalidixic acid (Nal: 0.25 to 128 mg/liter, 32 mg/liter) and tetracycline (Tet: 0.06 to 32 mg/liter, 16 mg/liter). The CLSI break points were used for all the antimicrobials except erythromycin. For the erythromycin, the National Antimicrobials Resistance Monitoring System (NARMS) break point was used.

Results

Prevalence of *Campylobacter*

The overall prevalence of *Campylobacter* in conventionally raised swine was 56.1%, while 54.6% in antibiotic free farms Table 1. Although a higher frequency of *Campylobacter* detected in conventional farms than ABF, this difference was not statistically significant ($p>0.05$). The pig level *Campylobacter* prevalence in the three states was 54.2%, 54.1%, and 58.17% in North Carolina, Ohio and Wisconsin, respectively. There was no significant difference in the prevalence of *Campylobacter* between the two production system in North Carolina (53% for conventional and 55.8% for ABF) and Ohio (51.9% for conventional and 59.5% for ABF) ($p > 0.05$). However, in Wisconsin significantly higher prevalence of *Campylobacter* was observed in conventional (70.6%) than ABF (51.5%) ($p<0.05$).

At slaughter, there was significantly higher recovery of *Campylobacter* at post-evisceration stage than pre-evisceration in both production systems. At the post evisceration stage, there was significantly higher recovery rate of *Campylobacter* from carcasses raised in conventional production (42.4%) than ABF (26.6%). The recovery was significantly reduced after chilling stage regardless of the method used (USDA or single swipe carcass swabbing method). The recovery rate of *Campylobacter* in the post chill stage was 3.4% and 3% using the USDA and single swipe methods, respectively.

Antimicrobial resistance

A total of 2360 *Campylobacter* isolates (1181 from conventional and 1179 from slaughter) were tested for susceptibility using six antimicrobials. At the farm level, resistance to all the six antimicrobials was observed with different frequency in both production systems. Frequency of resistance to tetracycline and erythromycin was significantly higher in conventional farms (72.1% for tetracycline and 69.5% for erythromycin) than ABF farms (60.2% for tetracycline and 37.5% for erythromycin) ($p<0.05$). Sixteen out of 849 from conventional and 83 out of 841 Campylobacter

isolates from ABF were resistant to ciprofloxacin. We have found higher frequency of resistance to ciprofloxacin and Nalidixic acid in ABF farms ($p<0.05$) Table 1. Resistance to gentamicin was not observed at the farm level in both conventional and ABF farms from North Carolina. At slaughter, however, resistance to gentamicin was observed at post evisceration and post chill stage. Multidrug-resistant *Campylobacter* strains were detected in both conventional and ABF herds. Twenty four percent of the *Campylobacter* isolates from North Carolina, 25% from Ohio and 20% from Wisconsin were pansusceptible. In North Carolina and Ohio isolates, the predominant resistance pattern observed was erythromycin–tetracycline followed by tetracycline. On the other hand, tetracycline was the predominant resistance pattern (23.5%) followed by erythromycin–tetracycline (16.6%) Fig 1.

	Production Stage	Production System	Pig/Carcass Tested	% Prevalence	# of Isolates Tested	Resistance profile Chl (%)	Ery (%)	Gen (%)	NA (%)	Cip (%)	Tet (%)
Farm	Finishing	Conventional	798	56.1	849	15 (1.8)	590 (69.5)	17 (2.0)	104 (12.3)	16 (1.9)	612 (72.1)
		ABF	702	54.6	841	14 (1.7)	315 (37.5)	21 (2.5)	191 (22.7)	43 (5.1)	506 (60.2)
Slaughter	Pre-Evisceration	Conventional	245	19.6	111	4 (3.6)	85 (77)	0 (0)	5 (4.5)	0 (0)	66 (59.5)
		ABF	251	21.9	143	21 (14.7)	50 (35)	0 (0)	26 (18.2)	4 (2.8)	64 (44.8)
	Post-Evisceration	Conventional	231	42.4	199	7 (3.5)	112 (56.3)	1 (0.5)	3 (1.5)	1 (0.5)	151 (75.9)
		ABF	259	26.6	165	14 (8.5)	79 (47.9)	2 (1.2)	13 (7.9)	2 (1.2)	106 (64.24)
	Post-Chill (USDA)	Conventional	244	3.7	11	0 (0)	6 (54.5)	0 (0)	0 (0)	0 (0)	6 (54.5)
		ABF	228	3.1	15	0 (0)	6 (40)	0 (0)	0 (0)	0 (0)	6 (40)
	Post-Chill	Conventional	245	2.4	11	1 (9.1)	10 (90.9)	1 (9.1)	0 (0)	0 (0)	7 (63.6)
		ABF	227	3.5	15	3 (20)	2 (13.3)	0 (0)	0 (0)	0 (0)	7 (46.7)

Tabel1. Prevalence and antimicrobial resistance profile of *Campylobacter* from different production systems and stages.

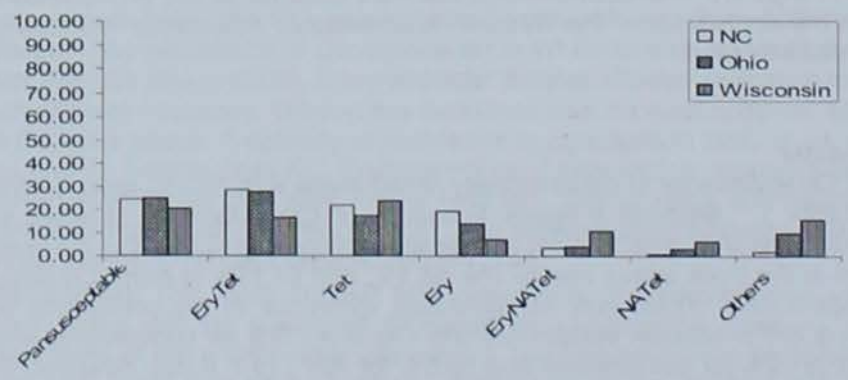

Fig1. The predominant *Campylobacter* resistance pattern observed among the three states

Discussion

The present study revealed that *Campylobacter* is prevalent in both conventional and antibiotic free swine farms. A *Campylobacter* prevalence of 56.1% was observed in conventional swineherds and previous reports indicated a prevalence ranging from 57.8 to 100% (Saez et al., 2000; Thakur and Gebreyes, 2005). However the prevalence was not significantly different from Antibiotic free farms (54.6%). There are limited information regarding the prevalence and antimicrobial resistance of *Campylobacter* in ABF swine farms. Luangtongkum and colleagues in 2006 compared the effect of production system on *Campylobacter* prevalence and antimicrobial resistance in poultry farms and showed high prevalence of *Campylobacter* in both conventional and organic poultry farms. There was no significant difference in the prevalence of *Campylobacter* among the three states. Higher recovery of *Campylobacter* at post evisceration stages could be due to cross-contamination during slaughtering. Chilling resulted in significant reduction in carcass *Campylobacter* load. In our study antimicrobial resistance to erythromycin and tetracycline were common. Different study in different

part of the world showed higher *Campylobacter* resistance to tetracycline and erythromycin from pig isolates (Guévremont et al., 2006; Mayrhofer et al., 2004; Bywater et al., 2004). The current finding indicated that higher frequency of resistance to ciprofloxacin and Nalidixic acid in ABF farms than conventional. The finding of resistance to erythromycin and ciprofloxacin is a concern to public health as these drugs are the drugs of choice in severe and complicated cases. This study highlights the high prevalence of antimicrobial-resistant *Campylobacter* in both conventional and ABF pig production systems.

References

1. Bywater R, H. Deluyker, E. Deroover, A. de Jong, H. Marion, M. McConville, T. Rowan, T. Shryock, D. Shuster, V. Thomas, M. Valle, and J. Walters. 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother.* 54(4):744-54.
2. Centers for Disease Control and Prevention. 2006. Preliminary FoodNet data on the incidence of infection with pathogens transmited commonly through food—10 states, United States,2005. *MMWR* 55(14);392-395
3. Engberg J, F.M. Aarestrup, D.E. Taylor, P. Gerner-Smidt, and I. Nachamkin. 2001. Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates. *Emerg Infect Dis.* 7:24–34.
4. Guévremont E., E. Nadeau, M. Sirois, and S. Quessy. 2006. Antimicrobial susceptibilities of thermophilic Campylobacter from humans, swine, and chicken broilers. *Can J Vet Res.* 70(2):81-6.
5. Gupta A., J.M. Nelson, and T.J. Barrett. 2004. Antimicrobial resistance among Campylobacter strains, United states, 1997–2001. *Emerg Infect Dis* 10:1102–9.
6. Luangtongkum T., T.Y. Morishita, A.J. Ison, S. Huang, P.F. McDermott, and Q. Zhang. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of Campylobacter spp. in poultry. *Appl Environ Microbiol.* 72(5):3600-7.
7. Mayrhofer S, P. Paulsen, F.J. Smulders, and F. Hilbert. 2004. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int J Food Microbiol.* 97(1):23-9.
8. Mead P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg Infect Dis.* 5(5):607-25.
9. National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals; approved standard, 2nd ed., M31-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. Prats G, B. Mirelis, T. Llovet, C. Munoz, E. Miro, F. Navarro. 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995–1998 in Barcelona. *Antimicrob Agents*
Chemother. 44:1140–1145.
11. Saenz Y., M. Zarazaga, M. Lantero, M.J. Gastanares, F. Baquero, and C. Torres. 2000. Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997–1998. *Antimicrob Agents Chemother.* 44:267–71.
12. Thakur S. and W.A. Gebreyes. 2005. Prevalence and antimicrobial resistance of Campylobacter in antimicrobial-free and conventional pig production systems. *J Food Prot.* 68(11):2402-10.